Scientific Abstract

With the advent of highly active anti-retroviral therapy (HAART), triple drug therapy comprising two nucleoside reverse transcriptase inhibitors in conjunction with either a protease inhibitor or non-nucleoside reverse transcriptase inhibitor has become the standard of care for HIV infection. However, a variety of factors can contribute to the failure of combination anti-retroviral therapies to durably suppress viral replication in HIV-1-infected patients. Patients who have a low $CD4^+$ T cell count or high plasma viral load before therapy is initiated are at high risk for subsequent virological failure. Patients that have been treated with sequential monotherapies in the past, have been infected with a highly resistant isolate of HIV-1 or who temporarily discontinue therapy as a "holiday" or because of drug intolerance are also at high risk for the anti-retroviral response to be only short–lived. In addition, life-long adherence to maintenance HAART will probably be required even in responding patients with undetectable viremia because of the reservoirs of latently infected cells that can persist for years. These limitations combined with the unknown long term durability and toxicity of these regimens have provided clear evidence that new and improved treatments for this chronic infectious disease are required.

Intracellular antibodies, termed intrabodies, may be useful for the gene therapy of HIV-1 infection. We have demonstrated the feasibility of using intrabodies to inhibit the function of the HIV-1 Tat protein, a critical regulatory protein of HIV-1 that is absolutely required for HIV-1 replication and for which anti-retroviral drugs are not available. Replication-defective retroviruses (MuLV) encoding a single-chain anti-tat intrabody have been used to *ex vivo* transduce PBMCs from three groups of HIV-1 infected individuals: three individuals immediately following a documented HIV-1 seroconversion illness, 23 individuals with asymptomatic HIV-1 infection (who had not had an AIDS-defining illness), and 22 individuals with AIDS. The results of these studies demonstrated that the sFvtat-transduced cells were significantly protected from the spread of HIV-1 infection, as compared to cells transduced with a control vector that does not express the anti-tat intrabody. We have also constructed MuLV vectors that express the phenotypic selection marker truncated human nerve growth factor receptor (ΔNGFR) alone (control vector) or with a humanized anti-tat sFv intrabody, termed sFvhutat2 and have prepared PG13 packaging cell lines and harvested GMP grade supernatants through the National Gene Vector Laboratories.

We propose to initiate an anti-tat intrabody clinical gene therapy trial in conjunction with the standard of care, HAART. Ten HIV-1-infected individuals that have $CD4^+ > 100mm^3$ and >1,000 copies/ml plasma HIV-1 RNA despite HAART will participate in the study. The two principal objectives of this pilot study are to assess the safety and adverse event profile of infusing autologous $CD8^+$T-cell depleted PBMCs that have been transduced *ex vivo* with retroviral vectors encoding ΔNGFR alone or with sFvhutat2 and to determine if there is an *in vivo* survival advantage of sFvhutat2-transduced $CD8^+$ T-cell depleted PBMCs within each patient in comparison to cells transduced with the control vector expressing ΔNFGR alone. The secondary objectives of this study are to perform further phenotypic, genotypic, virologic and immunologic studies on these two populations of cells after infusion through FACS selection and separation of cells expressing ΔNGFR alone or with sFvhutat2. We will also investigate whether cytotoxic T cell immune responses against the ΔNGFR and sFvhutat2- transduced cells are generated by the host *in vivo,* make preliminary observations on the effects of sFvhutat2 intrabody gene therapy on *in vivo* plasma viral RNA and $CD4^+$ T-lymphocyte levels and perform genotype and phenotype analyses on the HIV-1 Tat gene recovered from infected patient PBMCs before and after sFvhutat2 intrabody gene therapy to evaluate for changes in antibody binding affinity for Tat protein, changes in Tat protein-mediated transactivation of HIV-1 LTR promoter activity and for the potential development of neutralization escape mutants. These results will aid in guiding the design of new HIV-1 therapeutics that are directed against Tat protein and in our understanding of the consequences of altering the dynamics of the latent pool of HIV-1-infected cells.